Remarks

The Claims

Claims 24, 26, 27, 29-37, 44, 52, 54 and 61-66 are in the case.

Claims 22, 25, 28, 38, 39, 49-51 and 53 are canceled.

Claim 61 is the sole independent claim.

Claim 61 is amended herewith so that the subject matter of step (a) is simplified and so that the subject matter of steps (b)-(d) is explained. So far as step (a) is concerned, claim 61 as amended is based on alternative (ii) of claim 61 before amendment herein. As regards steps (b)-(d), claim 61 is amended to insert at the end of step (d) the phrase: --whereby a control is provided based on the body fluid--. Basis for this is found in the application as filed at page 18, lines 34-36. Please note that the investigation of a DNA (compare claim 54) such as a genomic DNA as defined in claims 27, 29, 30, 31 and also of cancer-associated nucleic acids such as those defined in claims 32-36 in the cancer cell fraction, would usually be meaningless without comparing to said control.

Claims 64 and 65 are new claims.

Claim 64 covers the subject matter of alternative (iii) of claim 61 before amendment herein. For clarity, step (a) of claim 61 does not require (but does not exclude either) a prior enrichment of disseminated cancer cells.

Claim 65 defines the plurality of cells in a case where the body fluid is blood. Support for this subject matter in the application as filed, is found, for instance, at page 17, lines 24-32, page 18, lines 30-31, and page 21, line 34 through page 22, line 1. Claim 65 recites cases where a plurality of cells of step (a) of claim 61 has not been subjected to removal of cancer cells from non-cancer cells.

Basis for new claim 66 is found in the application as filed at page 5, lines 15-24.

Attached as Appendix I, for ease of reference, is a consolidated version of the claims as amended, as if the application has been filed with the amended subject matter.

The Invention

Claim 61, as amended, is directed to a method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasized cancer cell in a body fluid from which metastatic cancer may arise. There is evidence that metastatic characteristics are acquired after cells have left the site of a primary tumor and that there are genetic differences between disseminated cells and primary tumor cells. Thus strategies that target cells in a primary tumor are unlikely to eradicate cells that have left the primary tumor and might later become seeds of metastatic growth. It follows that the hunt for therapeutic approaches should target disseminated (or micrometastasized) cancer cells. See Skipper, M., Nature, Vol. 4, p. 488, July 2003, copy attached. The instant invention allows determining the need for targeting for therapy, disseminated cancer cells from which metastatic cancer will arise. It follows that the claimed invention has lifesaving potential.

Please note that the investigations of claim 61 as amended are directed to determination from a body fluid and are much less invasive than investigations based on tissue samples.

Claim 61, as amended, requires two investigations, which for convenience we will respectively denote investigation (1) and investigation (2). Investigation (1) comprises step (a) of claim 61 as amended and is carried out with or without prior enrichment of disseminated cancer cells and with at least one cancer-related mRNA that is essentially not expressed in a non-cancer cell in the body fluid. Investigation (2) comprises steps (b)-(d) of claim 61 as amended and requires enrichment of cancer cells (step (b)) and investigation in isolated enriched fraction with a further nucleic acid that is different from the mRNA utilized for step (a) (see step (c) and "wherein" clauses) and requires comparison to a control based on the body fluid (step (d)). Since the cancer-related mRNA of investigation (1) is different from the cancer-related nucleic acid of investigation (2), the method of

claim 61 as amended qualifies as a multi-parameter analysis, with a second analysis including comparison to a control.

The Rejections

The only rejections are obviousness rejections.

As indicated above, claims 24, 26, 27, 29-37, 44, 52, 54 and 61-66 are in the case.

Claims 24, 26, 27, 32, 36-37, 44, 54, and 61-63 are rejected under 35 U.S.C. 103(a) as being obvious over Ditkoff et al. (Surgery, Vol. 120, 959-965), in view of Duffy, U.S. 5,871,917, in view of Hoon et al., U.S. 6,057,105.

Claims 29-31 and 33-35 are rejected under 35 U.S.C. 103(a) as being obvious over Ditkoff et al., in view of Duffy, in view of Hoon et al., further in view of Schmitz et al., U.S. 6,190,870.

Claim 52 is rejected under 35 U.S.C. 103(a) as being obvious over Ditkoff et al. in view of Duffy in view of Hoon et al., and further in view of Mitsuhashi, U.S. 5,978,797.

Reconsideration of all the rejections is respectfully requested.

Applicant relies below on the combination of Ditkoff et al. in view of Duffy in view of Hoon et al., being defective to make the sole independent claim (claim 61 as amended) obvious, so that all the claims remaining in the case are unobvious and allowable.

Response to the Rejection

The rejection takes the positions that:

(1) Ditkoff et al. teaches detecting circulating thyroid cells in peripheral blood (single parameter analysis).

(2) Duffy teaches detection of differentially methylated or mutated nucleic acid relative to the corresponding DNA from normal cells.

(3) Hoon et al. teaches a method of using multiple cancer markers to provide increased sensitivity over methods using a single marker.

The art combination of the rejection is defective firstly because it relies on unjustified overgeneralization of Hoon et al. (i.e., there is no basis in Hoon et al. for the generalization that is basis for the conclusion of unpatentability). Consider that Hoon et al. is limited to use of multiple markers in a first sample. On the other hand, the overgeneralization applies the disclosure of Hoon et al. to embrace using markers in respect to two samples at least one of which is enriched.

The art combination of the rejection is defective secondly because it has to rely on Duffy teaching detection of disseminated cancer cells from a body fluid and Duffy does not do this. Rather, Duffy investigates for $CD5^+$ B-type lymphocytes isolated from patients who have chronic lymphocytic leukemia (which do not qualify as disseminated cancer cells or micrometastasized cancer cells), i.e., cancer cells that have become detached from a primary tumor (compare page 5, lines 15-18 of the application as filed), or a tissue sample. On the other hand, claim 61 as amended, at least implicitly requires the possibility of disseminated cancer cells in a body fluid (see preamble and concluding portion).

The art combination of the rejection is defective also for the following reasons:

(1) The applied references do not teach detecting a plurality of cancer markers, each on a different sample while claim 61 requires this.

(2) The applied references do not teach detection of multiple cancer markers, at least one in an enriched sample, while claim 61 requires this.

(3) The applied references teach comparing to a control only to verify results on a first marker in one sample, not in respect to a second marker in a second sample. (Claim 61).

(4) When detecting compared to control, there is no teaching in the applied references of detecting risk of disseminated cancer cells or micrometastasized cancer cells from a body fluid whereas claim 61 requires this.

Moreover, even if one mistakenly assumes that Hoon et al. motivates combination of Ditkoff et al. and Duffy, as indicated above, required elements of claim 61 which are not obvious are missing, and furthermore the teachings of Ditkoff et al. and Duffy don't mesh to make claim 61 as amended obvious. The method of Ditkoff et al. requires a different sample processing from the method of Duffy. The preparation of a sample for being analyzed in accordance with the method of Ditkoff et al. is rather simple. The method of Duffy, however, requires the isolation of cancer cells and thus involves much more complex processing. Moreover, according to the method of Duffy, at least two differently processed samples have to be investigated for the same nucleic acid whereas the method of Ditkoff et al. works perfectly with a single sample. Moreover, according to the method of Duffy, a liquid sample does not have the potential of containing disseminated cancer cells.

Furthermore, the rejections ignore that the method of amended claim 61 provides a result not suggested in the prior art for the detection of the second marker. Consider that the investigation

involving detecting the second marker does more than exclude false positives; it also establishes a risk profile. In this regard consider the analysis in Appendix II hereto.

See also the declaration of Professor Giesing (an inventor herein), attached, which is directed to showing that the present invention is more than a mere combination of two cancer methods. The declaration shows that the claimed method not only reliably detects disseminated cancer cells, but also provides further cancer-related information, namely information about the origin of the cancer, e.g., of epithelial origin, where this is not already known. Moreover the declaration shows that surprising synergism is obtained by combining both investigations, as follows. The cancer-related mRNA that is essentially not expressed in a non-cancer cell of the body fluid simply shows relatedness to some kind of tissue and indicates that said tissue is spreading such mRNA (usually in the form of cells) into the body fluid. Whether said mRNA is indeed derived from a tumor and therefore would indicate the presence of a disseminated or micrometastasized cancer cell, has to be confirmed by investigation (2). Investigation (2), however, requires the enrichment of disseminated or micrometastasized cancer cells. As a matter of fact, this will involve a selection of a subset of cancer cells, depending on the separation technique used. Surprising is that the disseminated cancer cells isolated do not always comprise the cancer cells which express said mRNA. As is known, disseminated cancer cells may be further dedifferentiated and thus no longer express said mRNA. Said differentiated cells are those which are relevant for the further development of the disease (and allow drawing up a risk profile) but they do not allow establishment of connection with the origin of spread. On the other hand, the disseminated cancer cells which express said mRNA, are often proven irrelevant for the further development of the disease (and therefore often fail to be prognostic), but they provide information on the origin of the source of the spread.

A Power of Attorney (8 forms) is enclosed which redirects mail to the undersigned's law firm. Entry of this promptly is requested so the undersigned can check progress on line.

The application has been pending for a long time. A different attorney is now involved, and the aim is to provide a simplified claim structure and a comprehensive convincing response to the rejections.

Allowance is requested.

Respectfully submitted,

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Case: M-38030-US
B&T Docket No. GIES3003

APPENDIX I

CLAIMS PROPOSAL (Consolidated Version)

1. (61): A method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasized cancer cell in a body fluid from a subject, comprising:

(a) investigating, in a plurality of cells from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, for at least one first nucleic acid selected from the group consisting of a cancer-specific mRNA and a cancer-associated mRNA, wherein the mRNA is essentially not expressed in a non-cancer cell in the body fluid;

(b) isolating from the body fluid at least one cancer cell according to a method for removing cancer cells from non-cancer cells;

(c) investigating at least one cancer cell isolated according to step (b) for at least one second nucleic acid selected from the group consisting of a cancer-specific nucleic acid and a cancer-associated nucleic acid; and

(d) investigating at least one non-cancer cell from the body fluid for at least one second nucleic acid that is investigated in step (c) whereby a control is provided based on the body fluid,

wherein said first and second nucleic acids are different, wherein presence of said first nucleic acid in the plurality of cells and an increased or decreased presence of the second nucleic acid in the cancer cell relative to the presence or absence of said second nucleic acid in the non-cancer cell from the body fluid indicate an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

2. (New 64): The method of claim 1 (61) wherein said step of investigating, in a plurality of cells from a body fluid of a subject, for the mRNA that is essentially not expressed in a non-cancer cell in the body fluid takes place without previous removal of cancer cells from the plurality of cells.

3. (New 65): The method of claim 2 (New 64) wherein the body fluid is blood and the plurality of cells is the buffy coat or a mononuclear cell fraction derived from blood.

4. (62): The method of claim 1 (61) wherein the first nucleic acid is a first cancer-specific mRNA and the second nucleic acid is a second cancer-specific nucleic acid.

5. (63): The method of claim 1 (61) wherein the first nucleic acid is a first cancer-specific mRNA and the second nucleic acid is a cancer-associated nucleic acid.

6. (54): The method of claim 4 or 5 (62 or 63) wherein the second nucleic acid is selected from the group consisting of DNA and RNA.

7. (24): The method of claim 6 (54) wherein the RNA comprises mRNA.

8. (37): The method of claim 1 (61) wherein the cancer cell is removed from the body fluid by a method selected from the group consisting of microfiltration, density gradient centrifugation and antigen-specific immunoadsorption.

9. (26): The method of claim 1 (61) wherein the mRNA that is essentially not expressed in a non-cancer cell in the body fluid comprises all or a portion of a transcript of a gene selected from the group consisting of a CEA gene, a CK20 gene, a MUC1 gene, a tyrosinase gene and a MAGE3 gene.

10. (44): The method of claim 1 (61) wherein the mRNA that is essentially not expressed in a non-cancer cell in the body fluid encodes an organotypical gene, and wherein the presence of at least one of said mRNA encoding an organotypical gene indicates the type of malignant disease from which the cancer cell is derived.

11. (27): The method of claim 6 (54) wherein the DNA that is detected comprises genomic DNA selected from the group consisting of genomic DNA comprising a genomic mutation, genomic DNA comprising a gene that has undergone amplification, genomic DNA comprising a gene that has undergone loss of heterozygosity, genomic DNA comprising a translocated gene and genomic DNA comprising a gene polymorphism.

12. (31): The method of claim 11 (27) wherein the genomic DNA comprises all or a portion of a gene selected from the group consisting of a p53 gene, an erb-B2 gene, a c-myc gene, a K-ras gene, an RB gene, an APC gene and a DCC gene.

13. (29): The method of claim 6 (54) wherein the DNA is genomic DNA that comprises all or a portion of an oncogene.

14. (30): The method of claim 6 (54) wherein the DNA is genomic DNA that comprises all or a portion of a tumor suppressor gene.

15. (32): The method of claim 5 (63) wherein the cancer-associated nucleic acid comprises a coding portion of a gene selected from the group consisting of a tissue-specific gene, a metastasis-associated gene, a steroid hormone receptor gene, a drug resistance gene, an immunomodulation gene, a cell proliferation gene and an apoptosis gene, or a complementary nubcleic acid thereto.

16. (33): The method of claim 15 (32) wherein the metastasis-associated gene encodes a gene product selected from the group consisting of an aniogenesis factor, a motility factor, a growth factor, a matrix degradation factor and an adhesion factor.

17. (34): The method of claim 16 (33) wherein the matrix degradation factor is selected from the group consisting of a proteinase and a proteinase inhibitor.

18. (35): The method of claim 16 (33) wherein the adhesion factor is an adherin.

19. (36): The method of claim 7 (24) wherein the mRNA encodes a gene product selected from the group consisting of bFGF, bFGF-R, VEGF, VEGF-R1, VEGF-R2, MMP2 and TIMP3.

20. (52): The method according to claim 1 (61) wherein steps (a) – (d) are performed before and after administering a candidate anticancer therapy to a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell.

21. (66): The method of claim 61 where the disseminated or micrometastasized cancer cell originates from a primary tumor.

APPENDIX II

Amended Claim 61 Establishes a Risk Profile

This may be illustrated by the following situation:

(I) There are two patients having surgically treated breast cancer. In patient 1 no regional lymph node metastases are found (N0) while patient 2 shows metastatic involvement of regional lymph nodes (N1). Accordingly, one would expect patient 1 to have a better prognosis than patient 2.

(II) The blood of the first patient is investigated for a first mRNA that is essentially not expressed in a non-cancer cell in blood (step (a) of claim 61). The result is negative and, in accordance with Ditkoff et al., one has to presume that there are no disseminated cancer cells in the blood of patent 1.

The blood of the second patient is investigated for the same mRNA that is essentially not expressed in a non-cancer cell in blood (step (a) of claim 61). The result is negative and, in accordance with Ditkoff et al., one has to presume that there are disseminated cancer cells in the blood of patient 2.

(III) In accordance with Hoon, the blood of both patients is not investigated for an additional mRNA that is essentially not expressed in a non-cancer cell in blood (step (a) of claim 61). For both patients, the result is positive. This confirms Hoon et al. insofar as disseminated cancer cells are heterogeneous and therefore the investigation of more than one mRNA assists in detecting disseminated cancer cells with enhanced sensitivity, as the example of patient 2 shows.

(IV) When investigated in accordance with the steps (b)-(d), disseminated cancer cells of patient 1 show more genomic imbalances (such as genomic amplifications or LOHs) than disseminated cancer cells of patient 2. Accordingly, patient 2 would be expected to have a better prognosis than patient 1, which however is contrary to the expectations based on the TNM system as well as on the mRNA analysis.

In such a situation, the clinical follow-up, according to the experience of Prof. Giesing, will likely confirm the prognosis based on the results that were obtained with the second type of investigation of claim 61, e.g., the recurrence-free interval for patient 1 will be shorter than for patient 2.

For instance, the examples 5 and 6 of the present application show that a patient with positive results for several mRNAs that are essentially not expressed in a

non-cancer cell in blood (CK20, CEA, MUC1) can nevertheless have a better risk profile (low potential for metastasis; no evidence of drug resistance before chemotherapy) than the same patient with less positive results for mRNAs that are essentially not expressed in a non-cancer cell in blood (negative for CK20, CEA) but first signs of an ability to metastasize (bFGF-R-, VEGF-, VEGF-R1-mRNA positive after chemotherapy). Here, the ability to metastasize has been determined according to steps (b)-(d), i.e., investigation 2 of claim 61.